Chapter 3
Experimental Forward Mandibular Displacement in Sheep
3.1. INTRODUCTION

In the treatment of class II malocclusion, growth modification in the temporomandibular joint (TMJ) by the use of functional appliances is of great interest to orthodontists (see Chapter 1). Currently, knowledge of the responses of TMJ components to functional appliance treatment is mainly derived from animal experiments. While radiography, computer tomography or magnetic resonance imaging can be performed on human subjects, specific histological evaluation is denied. Thus, in order to more extensively investigate the adaptability of the TMJ complex to procedures that mimic orthodontic treatment regimens, specific functional appliances have been fitted to various experimental animals, including non-human primates and rodents, to prompt the mandible into a protrusive position.

Non-human primates and humans have similar anatomy with respect to the cranial base, upper face and mandible, as well as the TMJ (Isaacson et al., 1990). The appliances used for experimental mandibular displacement in non-human primates are also similar to those used in humans, such as an inclined plane and the Herbst appliance. Reproducible morphological changes in non-human primate TMJs after treatment have been variously reported. They include thickening of the posterior mandibular condylar cartilage together with increased bone deposition both on the posterior border of the ramus and on the anterior surface of the postglenoid spine (Stockli & Willert, 1971, McNamara & Carlson, 1979; McNamara et al., 1982; Hinton & McNamara,
1984; Woodside et al., 1987). However, high cost and their relative unavailability make the widespread use of this animal model difficult.

These issues apply less to rodents and a number of investigations have reported the results of mandibular protrusion experiments in those animals. Regarding the main objective of this study, which concerns the questions relating to bone metabolism, using rodents as the experimental animal may be beneficial because many studies investigating bone metabolism use rodents and that knowledge may be helpful to this present investigation. However, aside from the obviously marked anatomical differences between the TMJs of rodents and humans, discrepancies in the adaptive response of the TMJ after treatment have been reported in rodents. For example, some studies have shown thickening of the mandibular condylar cartilage in the posterior region of the condyle (Charlier et al., 1969; Petrovic et al., 1975) while others report thinning of this cartilage (Ghafari & Degroote, 1986). Tsolakis and Spyropoulos (1997) have suggested that inconsistent outcomes in such observations are a result of the anatomical characteristics of the dental arch and mandible as well as the design and fit of the appliances. These inconsistent morphological changes in the TMJ may lead to confusion when bone metabolism in the TMJ is studied.

As an alternative to expensive non-human primates and rodents which provide inconsistent outcomes, sheep have been previously used as an animal model in craniofacial growth research (Prince et al., 1997) and in oral and maxillofacial surgery research (Karaharju-Suvanto et al., 1990; 1996).
Although some differences in function exist, sheep have TMJs of similar size and anatomy to humans (Bosanquet & Goss, 1987). Based on this similarity, the sheep TMJ has been used as a model to evaluate diagnostic techniques (Kuirata et al., 1994) and treatment approaches (Bosanquet & Goss, 1989) for several TMJ disorders, e.g. degenerative pathology of the TMJ, termed as osteoarthritis in humans (Ishimaru & Goss, 1992). Using the sheep as a model, the progress of induced pathological conditions has been studied, e.g. disc perforation (Bosanquet et al., 1991a) and occlusal loss (Ishimaru et al., 1994). The treatment of TMJ disorders have also been studied in sheep where modalities including silastic replacement following discectomy (Bosanquet et al., 1991b) and fascia repair (Bosanquet et al., 1991c) have been investigated.

The purpose of this present investigation was to evaluate the sheep as a model for dentofacial orthopaedic research. This chapter describes a novel appliance, which produces mandibular displacement in sheep and presents findings related to TMJ adaptation.

### 3.2. RESULTS

#### 3.2.1. Procedure validation

A successful animal experimental procedure should cause less interference to growth and chewing pattern in the experimental animal. The growth of the animals was evaluated by weight gain and growth of the metacarpus of the animals.
The weight for each animal during the experiment is presented in Figure 3.1. The experimental animals maintained weight within the normal range for their age (Butterfield, 1988). The measurement error for growth of the metacarpus was 0.09 mm. No differences in the weight gain and metacarpus growth between the groups were detected (Table 3.1).

The operation period for inserting the implants varied from 1 hour to 4 hours 10 minutes with the median being 2 hours 30 minutes. The average operation time was 2 hours 32 minutes (SD: 1 hour 22 minutes). The number of occasions for repeated anaesthesia varied from 3 to 8 occasions with the median being 4 occasions.

Even though the operation time ranged from 1 to 4 hours 10 minutes, no correlation between the operation time and the weight gain was observed (Pearson correlation r=0.101, p=0.813). Furthermore, no correlation between the weight gain and the times of repeated anaesthesia was found (r=-0.263, p=0.529). Due to the lack of other information on sheep metabolism that has been monitored pre- and post-anaesthesia and the small sample size in this study, the present data was adopted to indicate that operation time and times of repeated anaesthesia had no relevant effect on the growth of the animals.

The chewing of the animals was evaluated by observation on a daily basis. Minor difficulties in chewing by the experimental animals were noticed within the first 2 or 3 days after placement of the appliance. These difficulties included irregular jaw motion and reduced chewing efficiency resulting in an
extended feeding time. Nevertheless, the animals grew and functioned normally.

Even though some components of the appliances did become displaced (11/16), they were promptly repaired and/or replaced (in 3 animals at < 1 week and 1 animal at < 2 weeks). However, it was observed that the mandible was still displaced forward during chewing when both of the lower components and at least one upper component were in place.

Figure 3.1. The weight of the animals during the experiment. The legend indicates the ID number of the animals in both the control and the experimental group. Negative values in time represent the pre-treatment observation period. Protrusive appliance group: #1, #5, #6 and #8. Inactivated appliance group: #4 and #7. Control group: #2, #3, #9 and #11.
Table 3.1. Weight gain and metacarpus growth of the animals in the control group and the experimental group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial Weight (kg) (17 wk-old)</th>
<th>Final Weight (kg) (32 wk-old)</th>
<th>Weight Gain (kg)</th>
<th>Metacarpus Growth (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
</tr>
<tr>
<td>Control (N=4)</td>
<td>27.1 4.59</td>
<td>32.4 3.64</td>
<td>5.3 2.50</td>
<td>6.60 1.22</td>
</tr>
<tr>
<td>Experimental (N=4)</td>
<td>29.9 2.02</td>
<td>31.9 3.20</td>
<td>2.0 3.39</td>
<td>6.08 0.86</td>
</tr>
</tbody>
</table>

- No significance was found between the two groups by two-sample t-test.

3.2.2. Observations from dental casts

Based on observations from the dental casts, the control animals had a normal tooth wear pattern with sharp cusps in one arch interlocked with the ridges in the opposite arch. The experimental animals had a flat occlusal surface induced by the appliance (Figure 3.2) creating a cusp-against-cusp occlusion.

![Figure 3.2](image)

Figure 3.2. Tooth wear pattern shows differences between the control and experimental animals.
Left: Normal tooth wear pattern with sharp cusps (control). 
Right: Flat occlusal surface (experimental).

3.2.3. Observations from cephalograms

By superimposing the tracings of cephalograms taken before and immediately after the placement of the appliance, downward and forward displacement of
the mandible was observed in the experimental animals (Figure 3.3). Detailed analysis of the effects of displacement is the subject of continuing investigation, the preliminary results of which are described in Chapter 7.

Figure 3.3. Functional appliance effects in sheep. Upper left: Lateral cephalometric radiograph of a sheep wearing the appliance; Upper right: Dot line shows the mandible at rest position; Lower left: Dot line shows the position of the mandible when occluding; Lower right: schematic presentation of the effectiveness of the appliance; arrow demonstrates the displacement of the mandible.

3.2.4. Histological Investigation

Microscopic observations of the histology of the TMJ revealed the following adaptive responses to the insertion of functional appliances.
Figure 3.4. Ramal dimorphisms. Upper: Straight ramus-control. Lower Left: Concave flexure in the ramus-experimental (arrow). Lower right: Convex flexure in the ramus-experimental (arrow) (×1 objective, von Kossa/H&E stain).
Mandibular Condyle. The condylar process was less tapered and rounder in the experimental group than in the controls. A flexure (Loth & Henneberg, 1996) in the posterior border of the ramus was evident on at least one side of the mandible in each of the animals. Such flexure was either convex or concave in form (Figure 3.4).

The condylar cartilage covering the superior bony layer of the condyle comprised a hypertrophic cartilage layer (hypertrophic zone), a proliferative cell layer (proliferative zone) and a fibrous articular layer (articular zone). In the control animals, the condylar cartilage was thinnest in the anterior region and gradually thickened posteriorly. In three out of the four experimental animals, anterior thickening of the condylar cartilage was evident in at least one side of the condyle (Figure 3.5).

Figure 3.5. Mandibular condyle, disc and portion of fossa. Anteriorly thickened condylar cartilage in the experimental group. Left: Condylar cartilage-control (arrow). Right: Same region of the condylar cartilage-experimental (arrow) (×2 objective, von Kossa /H&E stain).

The proliferative zone and hypertrophic zone were distinguishable by the presence of dense spindle-shaped cells and intensely stained extracellular
matrix, respectively. In the experimental animals a thickening of both the proliferative and hypertrophic zones was found in the anterior region (Figure 3.6).

![Figure 3.6. The anterior region of the mandibular condylar cartilage of the sheep. Left: Control. Right: Experimental showing thickened proliferative and hypertrophic zones (×10 objective, von Kossa/H&E).](image)

<table>
<thead>
<tr>
<th></th>
<th>Control (N=8)</th>
<th></th>
<th>Experimental (N=8)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td><strong>Anterior (µm)</strong></td>
<td>401.3</td>
<td>58.8</td>
<td>538.3</td>
<td>170.9 *</td>
</tr>
<tr>
<td><strong>Intermediate (µm)</strong></td>
<td>543.3</td>
<td>74.2</td>
<td>531.7</td>
<td>138.7</td>
</tr>
<tr>
<td><strong>Posterior (µm)</strong></td>
<td>634.0</td>
<td>124.3</td>
<td>618.8</td>
<td>178.6</td>
</tr>
</tbody>
</table>

* P<0.05; t test for two independent samples.

The condylar cartilage thickness was found to be significantly increased in the anterior region in the experimental group when measurements for both left and right sides were pooled for analysis (Table 3.2). The measurement error for condylar cartilage thickness was estimated to be 7.8 µm.
Posterior Wall of the Glenoid Fossa. The posterior wall of the glenoid fossa consists of a superficial layer of articular tissue and compact bone enveloping the underlying bony trabecular meshwork. The normal structure in control animals comprises a thin compact bony layer in front of the marrow cavity with superficial islands of newly formed bone parallel to the articular surface. A difference was observed on the anterior aspect of the compact bone layer in the experimental animals where a thick compact bone layer was observed in the experimental animals with merging or merged islands of newly formed bone (Figure 3.7).

3.3. DISCUSSION

3.3.1. Functional appliance and the mandibular displacement: static analysis vs. dynamic analysis

In human subjects, functional appliances help to correct the Class II malocclusion either through relocating the mandible forward, increasing the growth of the mandible or inducing a re-direction in growth of the mandible (see Chapter 1). In sheep, a forward mandibular displacement was observed through superimposition of the cephalograms before and immediately after placement of the appliance. The quantity of the mandibular displacement achieved by the appliance associated with growth is described in Chapter 8. These measurements only represent the mandibular positions at fixed times while the cephalograms were taken, therefore they are referred to as static analysis of the mandibular position. The dynamic analysis of the mandibular
position modified by the functional appliances in this study referred to the mandibular position during chewing.

Figure 3.7. Adaptive response in the posterior wall of the glenoid fossa following insertion of the functional appliances. Left: The wall in the control animals (arrow). Right: Same region of the wall in the experimental animals showing thickened compact bone (arrow) (×2 objective, von Kossa/H&E stain).

Mandibular movement in sheep is primarily medio-lateral in character which enables the crushing and grinding of the food to break up cellulose fibres (Dovitch & Herzberg, 1968). Excursive movements and several anatomical characteristics, such as no maxillary incisors, do not allow appliances to be placed on the incisors. Anatomically, sheep have a wider upper arch than the lower arch which does not allow bilateral occlusion. In addition, sheep have an edentulous space of up to 9 cm between the mandibular incisor region and the molar teeth. Moreover, the symphysis of the mandible in young sheep allows free lateral and rotational movement of the lower jaw. Consequently,
an appliance placed on the incisors cannot create a forward displacement of the mandible during occlusion because there are no upper incisors and sheep can easily move the mandible laterally to avoid incisor guidance. Therefore, the forward displacement of the mandible in this study was created with the appliances placed on the molar segments. As a result, sheep had to protrude their mandible during mandibular excursion when the ramps of the upper and lower appliance components engaged.

The patterns of jaw motion can be observed by continuous video recording. However, the technical difficulty is the mobility of the animals. Effective video recording would need either restraint of the animal or the use of multiple video cameras. The limitations of restraining animals carry further biases in amassing; e.g. the animals may bite differently when they are restrained and cannot access the feed freely. Continuous restraint of the animal is both harmful from the viewpoint of animal husbandry and also involves ethical concerns.

Limitation of using multiple video cameras involves the determination of accuracy of the observation. In deed, the chewing pattern of sheep cannot be observed well from a distance especially when animals are not directly facing the video camera. Moreover, sheep generally eat with their heads down and the view of their jaw motion is obstructed. Thus, in this study the jaw motion of sheep was assessed only by visual assessment in this study.
3.3.2. Appliance retention and mandibular displacement

During the experimental period, 11 out of 16 components of the appliances became displaced at least once. Although it was observed that the mandible was still displaced forward during chewing when both of the lower components and at least one upper component were in place, the magnitude of mandibular displacement might have been changed. In addition, a partially displaced appliance may influence the pattern of jaw motion. The jaw motion pattern may also have an important role in the outcome of functional appliance treatment with the underlying mechanism of changed jaw muscle activity. Furthermore, after the appliances were repaired and put back, another change in the magnitude of mandibular displacement likely to have occurred.

The effect of change in the magnitude of mandibular displacement might be similar to reactivation of the appliance, as it is performed in human subjects to displace the mandible in a stepwise manner. However, any changes caused by the damage and/or the loss of the appliance in the present animal experiment could not be quantified. The changed magnitude of mandibular displacement could be measured using 3-D cephalometry, but this would require additional general anaesthesia.

3.3.3. Adaptations in the TMJ

Sheep have a flat mandibular condyle with the medio-lateral dimension being larger than the antero-posterior dimension. In this study, in order to standardise the location of the sagittal histological sections, the coronoid
process was used as a reference structure. With sectioning through the centre of the coronoid process, standardised sections of the condyle could be reliably obtained.

The posterior wall of the glenoid fossa is a feature which appears in the medial and central parts of the sheep TMJ. Viewed from the anterior aspect of the joint, the wall has a roughly triangular shape with its long side sloping laterally. As a result, the wall is usually not visible in parasagittal sections in the lateral part of the joint. By measuring the height of this structure, standardised sections for comparison of the fossa can be identified within consecutive parasagittal sections. Dramatic adaptive responses were induced in the TMJ. The changes in the glenoid fossa were very similar to those reported in non-human primates. Hinton and McNamara (1984) have described compact layers of newly-formed bone which was increased on the anterior surface of the postglenoid spine. In the present study, increased formation of new bone was observed on the posterior wall of the glenoid fossa which is analogous to that observed in the postglenoid spine in the primate.

The condylar cartilage in experimental sheep was found to thicken anteriorly. This observation concurs with the observations of Kantomaa (1984) on rabbits but differs from those of McNamara and Carlson (1979) on monkeys and of Charlier et al. (1969) on rats. However, in these studies two different approaches were used to induce similar inter-relational changes between the condyle and the fossa. One was to use intra-oral appliances to prompt the mandible to a protrusive position (Charlier et al., 1969; McNamara & Carlson,
The other was surgical fixation of the interparietal, temporoparietal and both lambdoidal sutures to gradually displace the fossa to a backward position (Kantomaa, 1984). In this later study, the anteriorly thickened condylar cartilage was interpreted to be a consequence of the posterior migration of the glenoid fossa. As a result, the anterior condylar surface no longer participated in the articular function, thus, leading the mesenchymal cells in this region to differentiate into fibroblasts and they contributed to the thickening of the fibrous layer.

In contrast to the maxillofacial skeleton of the rabbit, no prominent articular eminence exists in the TMJ of the sheep. The condyle moves excursively within the fossa during function. It remains uncertain if protrusion of the mandible induced by the appliance would change the location of the articulating surface of the condyle. In the present study, thickening of the condylar cartilage anteriorly involved both the proliferative zone and the hypertrophic zone. This suggested that the progression of the mesenchymal cell lineage remained unchanged in the experimental sheep. While the observed increased thickness might be the result of a changed rate of cell differentiation, the reason for thickening of the condylar cartilage remains unanswered.

Convex or concave flexures of the posterior border of the mandibular ramus were observed in experimental animals. Ramal concave flexure at the level of the occlusal plane has been recorded in humans by Loth and Henneberg (1996). These authors have suggested "creation of the flexure is likely to
result from a change in the size, strength, or angulation of the muscles of mastication, especially the masseter and medial pterygoid muscles, which attach just below the level of flexure on the ramus”. The concave flexure observed in the present study was well above the level of the occlusal plane. Convex flexure has not been reported in the human mandible. Further studies are required to assess the development of such dimorphism.

3.4. CONCLUSION

The results of the present study indicate that the sheep provides a valid model for studying growth modifications in the TMJ region after mandibular displacement. The appliance used in this study has been effective in inducing adaptive responses in the TMJ. Sheep were found to cope well with the experimental procedures. Relatively larger in size when compared with non-human primates and rodents, the sheep TMJ allows the application of stereological methods or other histomorphometrical methods for detailed quantitative analysis. A serious impediment to research in growth modification in the TMJ in humans is lack of the specific histological evaluation. The gross architectural and histological similarities of sheep and human TMJs afford us an opportunity for comparison that might allow for a better understanding of the changes likely to occur in humans where treatment involves appliances that induce mandibular displacement.